Analysis of Methylene Blue Reduction by Ascorbic Acid

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Introduction

Chemical kinetics is the study of properties of chemical processes such as reaction rates, mechanisms and transition states. The mechanism and rate of chemical reactions can be determined using UV-Visible spectrophotometry. In this application note, the reaction of methylene blue with ascorbic acid is monitored with a Thermo Scientific Evolution Array UV-Visible spectrophotometer. The rate constant is also determined using Thermo Scientific VISIONcollect software.

Background

The mechanism of a chemical reaction can be identified by measuring a change in the overall reaction rate caused by changes in the concentration of individual reactants. In the simple chemical reaction:

\[ A + B \rightarrow P \]

the reaction rate (\( V \)) is proportional to the concentration of reactants, such that:

\[ V = k[A]^\alpha[B]^\beta \]

In this rate expression, \( k \) is the rate constant, \( \alpha \) is the order of the reaction with respect to A, and \( \beta \) is the order of the reaction with respect to B. Thus, the overall order of the reaction is:

\[ n = \alpha + \beta \]

Methylene blue (MB+) is a water-soluble dye molecule. Under acidic conditions, it is easily reduced to the colorless hydrogenated molecule leucumethylene blue (LB+) by ascorbic acid (H2A) as shown in Figure 1. The stoichiometry of the overall reaction is 1:1 and is represented in the equation:

\[ \text{MB}^+ + \text{H}_2\text{A} \rightarrow \text{LB}^+ + \text{D} \]

Figure 1: Reaction Mechanism of methylene blue with ascorbic acid

A rate constant (\( k_{\text{exp}} \)) is determined experimentally by measuring the change in concentration of methylene blue at 665 nm. As a first approximation, the rate of disappearance of MB+ can be expressed as in Equation 1 below. By strategically varying the individual reactants, a reaction mechanism can be determined by calculating constants \( k_0 \) and \( k_1 \) in Equation 1 using the experimental results.1

\[ \frac{d[\text{MB}^+]}{dt} = -(k_0 + k_1[HCl])[[\text{H}_2\text{A}][\text{MB}^+]] \\
= -k_{\text{exp}}[\text{MB}^+] \]

Equation 1:
Experiment and Results

Stock solutions of methylene blue (4.0 x 10^-4 mol/L), ascorbic acid (0.5 mol/L) and hydrochloric acid (2.0 mol/L) were prepared. Fifteen sample solutions were prepared from these stock solutions according to the conditions defined in Table 1. A blank was prepared for each sample by mixing together all of the reagents in the corresponding sample except methylene blue. All measurements were performed immediately following the addition of methylene blue to the sample due to the fast nature of the reaction. Time-based Kinetics mode was programmed using the parameters shown in Figure 2.

The Relationship Between Rate Constant and Methylene Blue Concentration, [MB+] (Set A):

The change in absorbance of methylene blue at 665 nm, plotted as a function of time, is shown in Figure 3. A first order fit of the absorbance between 0 and 80 seconds was used to determine an experimental rate constant (k_{exp}) of 0.0616. The data collected for the remaining samples (A2-C5) shows a similar pattern and their rate constants are shown in Table 2.
One benefit of the Evolution™ Array™ is its ability to collect the entire spectrum during kinetics experiments. The full spectrum of methylene blue can be observed by clicking the full spectrum icon in Time Based Kinetics Mode in the VISIONcollect™ software. The individual spectra for sample A1 are shown in Figure 4. These spectra were also used to construct the decay curve shown in Figure 3.

As shown in Table 2, variations in $k_{\text{exp}}$ are small with respect to methylene blue concentration. The average value of $k_{\text{exp}}$ is approximately 0.0624. All of the measured values fall within a small range of this average value and do not exhibit a trend in variation as a result of a decrease in concentration. Thus, we can conclude that the initial concentration of methylene blue is not a significant factor in the rate equation.

**The Relationship Between Rate Constant and Ascorbic Acid Concentration, $[H_2A]$ (Set B):**

To determine the effect of ascorbic acid on the overall rate, the concentration of $H_2A$ was varied according to the conditions defined in Set B, Table 1. A plot of $k_{\text{exp}}$ against $H_2A$ concentration exhibits a linear relationship as shown in Figure 5. A linear fit of the data results in a slope of 1.9026 M$^{-1}$s$^{-1}$ with a $R^2$ value of 0.9983. The slope of this curve is the $k_{\text{exp}}$ and can be entered into Equation 1:

Equation 1: \[ k_0 + k_1[H_2A] = 1.9026 \text{ M}^{-1}\text{s}^{-1} \]

The concentration of HCl in this sample set is held constant at 0.2 M. Entering this value into the equation above yields Equation 2:

Equation 2: \[ k_0 + k_1(0.2 \text{ M}) = 1.9026 \text{ M}^{-1}\text{s}^{-1} \]

**The Relationship Between Rate Constant and HCl Concentration, $[HCl]$ (Set C):**

In the final set of solutions, the concentration of HCl was varied according to Set C, Table 1. Plotting $k_{\text{exp}}$ against HCl concentration illustrates the linear relationship of the rate with respect to the concentration of HCl (Figure 6). The resulting linear fit has a slope of 0.2212 M$^{-1}$s$^{-1}$ and a $R^2$ value of 0.9981. Taking the slope as the experimental rate and substituting it into Equation 1 we find:

Entering the concentration of $H_2A$ (0.045 M), the value for $k_1$ is determined as follows:

\[ k_1 = 4.92 \text{ M}^{-2}\text{s}^{-1} \]

Entering the value of $k_1$ into Equation 2, the value of $k_0$ is determined as follows:

\[ k_0 + (4.92 \text{ M}^{-2}\text{s}^{-1})(0.2 \text{ M}) = 1.9026 \text{ M}^{-1}\text{s}^{-1} \]

\[ k_0 = 0.92 \text{ M}^{-1}\text{s}^{-1} \]

**Experimental Rate Law**

The rate law is obtained by combining the experimental results from sets A-C. This rate law for the oxidation of methylene blue by ascorbic acid under acidic conditions is shown in Equation 3.

Equation 3: \[ \frac{d[MB^+]}{dt} = -[0.92 + 4.92[HCl]][H_2A][MB^+] = -k_{\text{exp}}[MB^+] \]
Conclusion

The analysis of methylene blue reduction by ascorbic acid is performed quickly and easily with the Evolution Array and VISIONcollect software. In this experiment, rate data was obtained for the analysis and rate constants were simultaneously calculated using the kinetic analysis function of the VISIONcollect software. The export function of the VISIONcollect software facilitates further data processing in EXCEL®. From this analysis it was determined that the rate constant of the reaction is dependent on the concentration of both ascorbic acid and HCl and not on the initial concentration of methylene blue.

References